

CHEMICAL EXAMINATION
OF
OENANTHE CROCATA

BY
FRANK TUTIN



THE WELLCOME CHEMICAL RESEARCH LABORATORIES
FREDERICK B. POWER, PH.D., LL.D., *Director*
6, King Street, Snow Hill
LONDON, E.C.

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The umbelliferous plant, *Oenanthe crocata*, Linné, occurs commonly on the banks of streams and in wet ditches in England and Western Europe, and has frequently attracted attention on account of its poisonous properties. The roots of this plant are in clusters, some of them being of a thick, tuberous nature, somewhat resembling the parsnip in appearance. Numerous cases of fatal poisoning have been recorded, both with human beings and with cattle, which have occurred through eating these roots. The above-mentioned species of *Oenanthe* has been described by Mr. E. M. Holmes (*Pharm. Journ.*, 1902 [4], 14, 431) as the most dangerous and virulently poisonous of all our native plants, since it usually causes death more quickly than aconite, and the tuberous roots are specially liable to be eaten, owing to their somewhat tempting appearance and not unpleasant taste.

A tincture of the plant is stated to be used by homœopathic practitioners in the treatment of certain forms of epilepsy, and the tubers have also been employed by herbalists for the cure of suppurating wounds.

The only chemical work of note on the subject is by J. Poehl (*Arch. exp. Path. Pharm.*, 1895, 34, 258), who obtained from the tuberous roots an amorphous neutral product, soluble in ether, which he designated "œnanthotoxin." Poehl assigned to "œnanthotoxin" the formula $C_{17} H_{22} O_5$ or $C_{33} H_{42} O_{10}$, but since it is of an indefinite character, and

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no evidence of its homogeneity can be obtained, no significance should be attached to the result of its analysis.

It was, therefore, deemed of interest to conduct a complete chemical examination of the plant, the results of which are embodied in the present paper. This has led to a confirmation of the conclusion arrived at by Poehl, that the toxic principle is a neutral resin, but a number of other substances have also been isolated.

Experimental.

The material employed for this investigation consisted of the entire plants of *Ænanthe crocata*, Linné, which had been specially collected early in February on the bank of the Thames at Kew. By far the greater part of the weight of the plants was represented by the thick, tuberous roots, but a number of fibrous roots and a crown of small, immature leaves were also present. The amount of the fresh plant collected was 164 kilograms, and this, after being sliced and dried, weighed 36·7 kilograms. The loss on drying was, therefore, equivalent to 77·7 per cent. of the original weight.

Test for an Alkaloid and an Enzyme.—20 Gms. of the finely ground tuberous roots were digested with Prollius' fluid, and the resulting liquid subjected to the usual tests for an alkaloid, but with a negative result. The young leaves, as well as the mature leaves and stems and the fully developed fruits of the plant, which were collected in summer, when separately tested, were also found to be devoid of alkaloid.

In order to ascertain whether an enzyme was present, a quantity (500 Gms.) of the fresh tuberous roots was crushed and mixed with sufficient water to cover them, and the mixture kept for several hours, after which the aqueous liquid was expressed, and filtered. On adding absolute alcohol to a portion of the liquid so obtained the amount of precipitate produced was so small as to indicate that not more than a trace of enzyme or gum was present.

Preliminary Extraction of the Plant.—A quantity (50 Gms.) of the powdered plant was extracted successively in a Soxhlet apparatus with various solvents, when the following amounts of extract, dried at 120°, were obtained :—

| | | | | |
|-------------------------------------|-----------|----------|---|----------------|
| Petroleum (boiling-point 33-50°) .. | extracted | 0·72 Gm. | = | 1·44 per cent. |
| Ether | „ | 0·65 | „ | = 1·30 „ |
| Chloroform | „ | 0·14 | „ | = 0·28 „ |
| Ethyl Acetate | „ | 0·30 | „ | = 0·60 „ |
| Alcohol | „ | 12·40 | „ | = 24·80 „ |
| Total | | 14·21 | „ | = 28·42 „ |

For the purpose of a complete examination, a quantity (36 kilograms) of the dried and ground *Enanthe crocata* plant was completely extracted by continuous percolation with hot alcohol. After the removal of the greater part of the solvent, a viscid, dark-coloured extract was obtained, which amounted to 13.55 kilograms.

DISTILLATION OF THE EXTRACT WITH STEAM. SEPARATION OF AN ESSENTIAL OIL.

A quantity (4 kilograms) of the above-mentioned extract, representing 10.62 kilograms of the dried plant, was mixed with water, and steam passed through the mixture for several hours. The distillate, which amounted to 7 litres, contained a quantity of yellow oil floating on the surface. It was extracted with ether, the ethereal liquid being washed, dried, and the solvent removed, when a quantity of an essential oil was obtained. This was distilled under 100 Mm. pressure, when it passed over between 100 and 200°, chiefly between 180 and 200°, as a pale yellow oil possessing a somewhat unpleasant odour. The amount of essential oil obtained was 10.5 Gms., being thus equivalent to about 0.1 per cent. of the weight of the dried plant. The essential oil gave a reaction indicating the presence of furfural, and had the following constants:— $d_{15}^{15} = 0.9381$; $n_D^{20} + 1^{\circ} 16'$ in a 25 Mm. tube.

NON-VOLATILE CONSTITUENTS OF THE EXTRACT.

After the distillation of the extract with steam, as above described, there remained in the distillation flask a dark-coloured aqueous liquid (A) and a quantity of a brown resin (B). The latter was collected and repeatedly washed, the washings being added to the main portion of the aqueous liquid.

EXAMINATION OF THE AQUEOUS LIQUID (A).

The aqueous liquid (A), which amounted to about 9 litres, was repeatedly extracted with ether, the ethereal extracts being united, washed with water, and concentrated to a small bulk. A large volume of petroleum was then added to the concentrated ethereal liquid, when a tarry product was precipitated. The clear liquid was then decanted, and the tarry matter dissolved in ether, after which it was separated into a number of products by systematic extraction with aqueous solutions of ammonium carbonate, sodium carbonate, and

potassium hydroxide. A portion of the material was of a neutral character, and was not removed by the alkalies. All of the various products obtained were examined, but were found to consist entirely of resinous substances, with exception of the material removed by 20 per cent. aqueous potassium hydroxide subsequent to extraction with a 5 per cent. solution of the alkali. The liquid obtained by treatment with the stronger alkali was acidified and the liberated product extracted with ether. The material so obtained consisted largely of a black, tarry product, but gave evidence of also containing a small amount of a crystalline substance. It was boiled with a large volume of very dilute alcohol and the tar removed by treatment with animal charcoal and filtering, when the clear liquid deposited, on cooling, a quantity of long, colourless needles. This substance, after recrystallisation from dilute alcohol, melted at 83° . It yielded no colour when treated with alkalies or with ferric chloride solution in the cold, but when heated with the latter a brown resinous precipitate was formed. The crystalline substance was immediately charred by cold concentrated sulphuric acid, and it appeared to be very unstable, for its solutions soon darkened on keeping, and the solid substance, particularly when exposed to light, gradually acquired a deep purple tint. The amount of the product available was not sufficient for analysis, and an attempt to isolate a further quantity by working up an additional amount (5 kilograms) of the original alcoholic extract resulted in failure, the substance having evidently become resinified on keeping.

The clear petroleum solution which had been decanted from the tarry product from which the above-described crystalline substance had been obtained was evaporated, the residue dissolved in ether, and resolved into a number of fractions by treatment with various alkalies. The greater part of the material was of a resinous nature, but the ammonium carbonate extract also yielded a product which crystallised from hot water in colourless needles, melting at 155° and yielding a violet colouration with ferric chloride.

0.0872 gave 0.1944 CO_2 and 0.0353 H_2O . C = 60.8; H = 4.5.

$\text{C}_7\text{H}_6\text{O}_3$ requires C = 60.9; H = 4.3 per cent.

This substance was thus identified as salicylic acid.

The original aqueous liquid (A), which had been extracted by means of ether, as above described, was thoroughly extracted with successive portions of amyl alcohol. The total

amount of material removed by this treatment was comparatively small. It was divided into two portions, one of which was readily soluble, and the other very sparingly soluble in dry amyl alcohol, but nothing definite could be obtained from either of these products.

The original aqueous liquid, after being extracted with amyl alcohol, was treated with a slight excess of a solution of basic lead acetate. A yellow precipitate was thus formed, which, however, when decomposed by hydrogen sulphide yielded only a small amount of brown amorphous product. The filtrate from the basic lead acetate precipitate was treated with hydrogen sulphide for the removal of the lead, and the filtered liquid concentrated to the consistency of a syrup. This was thoroughly examined, but appeared to contain only dextrose and lævulose. It was lævorotatory, readily yielded *d*-phenylglucosazone (m.p., 215°), and, on acetylation by means of acetic anhydride in presence of a little *d*-camphorsulphonic acid, gave β -penta-acetyl-dextrose (m.p. 130°). A subsequent observation, however, rendered it evident that the dextrose and lævulose had, to a considerable extent at all events, been formed by the hydrolysis of the cane sugar which is present in the plant.

EXAMINATION OF THE RESIN (B).

This resin was a dark, greenish-brown, viscid mass, and amounted to 310 Gms., being thus equivalent to nearly 3 per cent. of the weight of the dried plant employed. It was dissolved in alcohol, mixed with purified sawdust, and the dried mixture extracted successively in a Soxhlet apparatus with light petroleum (b.p. 35-50°), ether, chloroform, ethyl acetate, and alcohol.

PETROLEUM EXTRACT OF THE RESIN.

Isolation of Hentriacontane, C₃₁H₆₄.

The petroleum extract was a dark green, viscid oil, and amounted to 166 Gms. It was dissolved in about 200 C.c. of ether, and the mixture cooled, when a quantity of a solid separated. The latter was collected and heated with chloroform, when all but a small quantity of brownish material dissolved. This was found to consist largely of ipuranol, and was added to the further amount of this substance subsequently obtained. The material dissolved by the chloroform appeared to consist of a hydrocarbon. It was

distilled under diminished pressure, and the distillate crystallised from ethyl acetate, when it formed pearly leaflets, melting at 68° , and was identified as hentriacontane.

0.1390 gave 0.4321 CO_2 and 0.1845 H_2O . $\text{C}=84.7$; $\text{H}=14.7$.

$\text{C}_{31}\text{H}_{64}$ requires $\text{C}=85.3$; $\text{H}=14.7$ per cent.

Isolation of Triacontane, $\text{C}_{30}\text{H}_{62}$, and a Phytosterol.

The filtrate from the hentriacontane and ipuranol was diluted with ether, and the ethereal liquid shaken successively with aqueous ammonium carbonate, potassium carbonate, and potassium hydroxide. The total amount of material removed by this treatment was about 20 Gms., but it was found to consist entirely of amorphous products, with the exception of a small amount of fatty acids. The ether was accordingly evaporated from the neutral portion of the extract, and the residue, which was a dull green, viscid mass, hydrolysed by boiling for some time with an alcoholic solution of potassium hydroxide, when the mixture quickly acquired a dark reddish-brown colour. The alcohol was then evaporated, water added, and the alkaline liquid extracted with ether, the ethereal liquid being washed, dried, and evaporated, when a considerable amount of a very dark reddish-brown viscid residue was obtained. This was dissolved in a mixture of ethyl acetate and alcohol, when, on keeping, it deposited a quantity of crystalline material which was recognised as hentriacontane. The filtrate from this hydrocarbon was allowed to evaporate slowly, when a further amount of solid was deposited. This appeared to consist largely of hydrocarbon, but also gave colour reactions indicating it to contain some phytosterol. The mixture was then boiled for some time with acetic anhydride, the liquid being subsequently cooled and filtered, when it was found that a small amount of phytosteryl acetate, practically free from hydrocarbon, was contained in the filtrate. This compound, after several crystallisations, melted at 125° , and, on hydrolysis, yielded a phytosterol, which melted at 135° . The amount of this substance was not sufficient for further investigation, but its properties, and those of its acetyl derivative, rendered it very probable that it was sitosterol, $\text{C}_{27}\text{H}_{46}\text{O}$.

The hydrocarbon, which had been separated from the phytosterol by means of acetic anhydride, was fractionally crystallised many times from ethyl acetate, when it yielded

a further small amount of hentriacontane, but the greater part of it formed lustrous plates, melting at 65° , and was identified as triacontane.

0.1325 gave 0.4144 CO_2 and 0.1761 H_2O . $\text{C}=85.3$; $\text{H}=14.7$.

$\text{C}_{30}\text{H}_{62}$ requires $\text{C}=85.3$; $\text{H}=14.7$ per cent.

The original filtrate from the mixture of hydrocarbons and phytosterol would not yield any further amount of crystalline material. It contained a large amount of a viscid, dark reddish-brown product, which was undistillable, and from which nothing definite could be obtained.

Isolation of Ipuranol, $\text{C}_{23}\text{H}_{38}\text{O}_2(\text{OH})_2$.

The alkaline aqueous liquid which had been extracted with ether, as above described, was acidified and again shaken with ether. After a short time the mixture separated into three layers, the middle one of which consisted of an emulsion. This was separated from the aqueous and ethereal layers and washed many times with ether, after which it was deprived of this solvent by means of a current of air, and the precipitated solid collected on a filter. This substance amounted to about 0.5 Gm., and proved to be ipuranol, $\text{C}_{23}\text{H}_{38}\text{O}_2(\text{OH})_2$ (*Amer. Journ. Pharm.*, 1908, **80**, 251). It was mixed with the small quantity of the same substance previously obtained and crystallised from dilute pyridine with the employment of animal charcoal. Very small, colourless leaflets were thus obtained, which melted and decomposed at $285\text{--}290^{\circ}$, and gave a colour reaction identical with that afforded by ipuranol. On acetylation it yielded diacetyl-ipuranol (m.p. 161°), and on benzylation in pyridine solution it was converted into the dibenzoyl derivative, which melted at 196° .

Examination of the Fatty Acids.

The ethereal liquid which had been separated from the emulsion containing ipuranol, as above described, was washed, dried, and the solvent removed, when a quantity (25 Gms.) of fatty acids was obtained. These acids, together with the small amount of free acids previously mentioned, were converted into their methyl esters, and the latter fractionally distilled five times under 60 Mm. pressure, when the following fractions were obtained: below 240° ; $240\text{--}250^{\circ}$; above 250° . The last fraction was very small and yielded nothing

definite, whilst the greater part of the material was contained in the middle fraction. The fraction boiling below $240^{\circ}/60$ Mm., however, amounted to about 6 Gms., and, on keeping, solidified to a crystalline mass. It was freed from adhering oil by being drained on porous earthenware, when a crystalline ester, melting at 29° , was obtained, which was identified as methyl palmitate. On hydrolysis it yielded palmitic acid (m.p. 63°). The fraction of esters distilling between 240° , and $250^{\circ}/60$ Mm., which did not deposit any solid, was hydrolysed, and the resulting acids dissolved in ethyl acetate. The solution so obtained deposited a further quantity of palmitic acid, which was removed by filtration, but no evidence could be obtained of the presence of any other solid acid. The filtrate from the palmitic acid was evaporated, distilled under diminished pressure, and cooled, when a further quantity of the saturated acid separated and was removed by filtration. The filtrate consisted of liquid, unsaturated acids. An analysis and determination of the iodine value of the latter gave the following results:—

0.1109 gave 0.3121 CO_2 and 0.1140 H_2O . $\text{C}=76.8$; $\text{H}=11.4$.

$\text{C}_{18}\text{H}_{32}\text{O}_2$ requires $\text{C}=77.1$; $\text{H}=11.4$ per cent.

0.2052 absorbed 0.3886 iodine. Iodine value = 189.4.

$\text{C}_{18}\text{H}_{32}\text{O}_2$ requires iodine value = 181.4.

It thus appears that the unsaturated acids consisted chiefly of linolic acid.

ETHER, CHLOROFORM, ETHYL ACETATE, AND ALCOHOL EXTRACTS OF THE RESIN.

The ether extract of the resin was a brown, sticky solid, and amounted to 103 Gms. It was dissolved in ether and treated with various alkalis, but this treatment only removed about half of the material. The products obtained on acidifying the various extracts were all nearly black, amorphous resins, and nothing crystalline could be isolated from any of them, with the exception of a trace of an almost colourless crystalline substance, melting at $102-103^{\circ}$, which was separated from the ammonium carbonate extract. The neutral portion of the extract was a dull green, viscid mass resembling in appearance the corresponding product from the petroleum extract of the resin, and, like the latter, it quickly became dark reddish-brown in colour when heated with alcoholic potash. Nothing crystalline could be separated from it, and

the product obtained by heating it with alcoholic alkalis was only a neutral, viscid, undistillable mass.

The chloroform extract of the resin amounted to 31.7 Gms., and formed an almost black resin, from which nothing definite could be obtained. The ethyl acetate and alcohol extracts amounted to 1.57 and 4.22 Gms. respectively. They were almost black, amorphous solids, from which nothing crystalline could be isolated.

Isolation of Cane Sugar.

The original alcoholic extract of the plant, when kept for a few weeks, deposited a large amount of crystals. A quantity of it was stirred with alcohol, water being gradually added, until the liquid portion of the mixture was a fairly homogeneous, thin syrup. The crude crystals were then collected on a filter and washed, first with dilute alcohol and subsequently with ethyl acetate, after which they were dried. The material so obtained was found to be almost pure cane sugar, and the amount was equivalent to 3.8 per cent. of the weight of the dried plant. It was recrystallised from dilute alcohol, when, on heating somewhat rapidly, it melted at 188° .

0.1887 gave 0.2910 CO_2 and 0.1115 H_2O . $\text{C} = 42.0$; $\text{H} = 6.5$.

$\text{C}_{12}\text{H}_{22}\text{O}_{11}$ requires $\text{C} = 42.1$; $\text{H} = 6.4$ per cent.

A determination of the specific rotatory power gave the following result :—

2.0003, made up to 20 C.c. with water, gave $a_D + 13^{\circ} 22'$ in a 2-Dcm. tube, whence $[\alpha]_D + 66.8^{\circ}$.

Since no cane sugar could be detected in the aqueous liquid (A), it is evident that this compound must have become inverted during the steam distillation by the organic acids present.

PHYSIOLOGICAL TESTS.

In order to ascertain the source of the poisonous properties of the *Enanthe* root, a number of products obtained during the present investigation were kindly tested by Dr. H. H. Dale, director of the Wellcome Physiological Research Laboratories, to whom my best thanks may here be expressed. All the experiments were conducted with guinea-pigs, the preparations being administered *per os*.

The aqueous liquid (A) and the chloroform and alcoholic extracts of the resin (B), when administered in doses of 1 Gm.,

produced no effect. The petroleum and ether extracts of the resin, however, when given in similar doses rendered the animal hyper-excitabile in two to four hours after ingestion, and marked convulsions, with trismus, soon appeared. The heart-beat became very noticeably slow for a guinea-pig, and the convulsions persisted until the animal died.

In order to ascertain more exactly the nature of the active principle, the neutral and acidic portions of the ether extract of the resin were separately tested, when it was found that, although both preparations possessed some activity, the neutral one was very much the more active. It is evident, therefore, that the chief active principle of *Ænanthe crocata* is represented by the dull green, viscid, neutral product which formed a considerable proportion of both the petroleum and ether extracts of the resin. This neutral material corresponds to the "ænanthotoxin" of Poehl (*loc. cit.*). As already stated, this product becomes dark reddish-brown on treatment with caustic alkalis, but nothing crystalline could be obtained from it.

Since the toxic principle could not be obtained in a form suitable for perfusion or injection, its action could not be more fully investigated, but there was nothing in the evidence obtained to preclude the possibility that the poisonous principle of *Ænanthe* may resemble that of *Erythrophleum guineense*, Don, in its action, combining a digitalis-like effect on the heart with a picrotoxin-like action on the nervous system.

SUMMARY.

The material employed in this investigation consisted of the entire dried plants of *Ænanthe crocata*, Linné, which had been specially collected for the purpose in early spring, and therefore represented chiefly the tuberous roots.

The roots were found to be devoid of enzyme, and no part of the plant, at any stage of growth, contained an alkaloid.

An alcoholic extract of the plant, when kept for some time deposited an amount of crystalline cane sugar equivalent to 3·8 per cent. of the weight of dried material employed.

The above-mentioned alcoholic extract, when distilled in a current of steam, yielded a yellow essential oil possessing a somewhat unpleasant odour. This oil had the following constants:— $d_{15^\circ/15^\circ} = 0\cdot9381$; $\alpha_D + 1^\circ 16'$ in a 25 Mm. tube. From the portion of the alcoholic extract which was soluble in water there were isolated a small amount of a colourless

crystalline substance (m.p. 83°), which, on keeping, assumed a purple colour, and some salicylic acid. It also contained some amorphous products and a very large amount of dextrose and lævulose.

The portion of the extract which was insoluble in water consisted of a dark-coloured, viscid resin amounting to nearly 3 per cent. of the weight of the plant employed.

From this material the following compounds were isolated : —Triacontane, $C_{30}H_{62}$; hentriacontane, $C_{31}H_{64}$; a phytosterol (m.p. 135°); ipuranol, $C_{23}H_{38}O_2(OH)_2$; palmitic acid; and a mixture of unsaturated acids consisting chiefly of linolic acid. The greater amount of the material insoluble in water was, however, of a resinous nature. The neutral portions of the petroleum and ether extracts of this resin represent the toxic principle of the *Ænanthe crocata*.

To a similar neutral product Poehl (*Arch. exp. Path. Pharm.*, 1895, 34, 258) has previously given the name “ænanthotoxin” and assigned to it the formula $C_{17}H_{22}O_5$ or $C_{33}H_{42}O_{10}$. In view, however, of the total lack of evidence of the homogeneity of this product, and the great probability of its complex nature, it is evidently undesirable to give it either a name or a formula which would indicate it to be a definite compound.

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